Infectious Agents in Immunodeficient Murine Models: Pathogenicity of Actinomyces israelii Serotype I in Congenitally Athymic (Nude) Mice

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Nude (Nu/Nu) and heterozygous (Nu/+ ) mice were infected with Actinomyces israelii serotype I by either intranasal instillation or intravenous injection. Lung clearance data and 50% lethal dose values indicated that T-lymphocytes were not necessary for pulmonary clearance or prevention of systemic actinomycosis. However, T-lymphocyte deficiency may play a role in the localized cervicofacial form of actinomycosis.

Actinomyces israelii is frequently isolated from the oral cavity of humans, where it resides as a commensal organism (5). It only occasionally causes disease, and the conditions which lead to the organism’s invasiveness are not understood. Most reports in the literature indicate that A. israelii will not infect mice readily; however, there have been some studies of experimental infections in mice, usually after inoculation of clumps of bacteria (frequently with some form of adjuvant) into the host (3, 5).

Brown and von Lichtenberg (3) studied the pathogenesis of several strains of A. israelii in mice and ascertained that rough strains of this organism could infect healthy mice when large numbers of organisms were injected intraperitoneally. The pathology of the resultant lesions were similar to those described for the human form of Actinomycosis. Therefore, their work indicated that mice could be used as a model system for studying host-parasite interactions with this organism.

The mechanisms of host resistance and immunity to A. israelii are not known. However, because the disease caused by A. israelii may resemble disease caused by Nocardia, and the organisms may be mistakenly confused since they share the same basic morphology, it is important to differentiate the host response to Actinomyces and Nocardia. It was previously shown that the host resistance to N. asteroides involved an active T-lymphocyte system and probably cell-mediated immunity (1, 2). Lung clearance data (utilizing thiglycolate mice) of intranasally instilled Nocardia asteroides clearly established that T-cells are important in pulmonary clearance and prevention of dissemination. Similar studies using A. israelii have not been reported.

A. israelii serotype I (obtained from the California State Public Health Laboratories, Berkeley, Calif.) was grown in thiglycolate broth for 48 h at 37°C. The cells were centrifuged at approximately 2,000 × g for 15 min at room temperature, and the pellet was suspended in 0.85% sterile saline. Cell suspensions were diluted and used to infect mice. At the same time, plate counts were made utilizing brain heart infusion agar plates and incubated for 1 week anaerobically in GasPak jars at 37°C. The cell suspensions were either injected into the tail vein (i.v.) or administered intranasally (i.n.) into athymic (Nu/Nu) or heterozygous (Nu/+ ) littermates (1, 2). Immediately after i.n. administration, five mice from each group were sacrificed by an overdose of ether, and the left lung was removed. It was placed in 3 ml of thiglycolate broth and homogenized as previously described (2). Plate counts utilizing anaerobic jars were made to assess the relative numbers of bacteria taken into the lung. These procedures were repeated at specific time intervals to determine lung clearance. The 50% lethal dose values were determined for each route of inoculation in both N:NIH(S) athymic (Nu/Nu) and heterozygous mice (Nu/+ ) as previously described (1, 4).

Kill curve and lung clearance data were obtained for a period of 3 months after infection. Three of 23 nude mice infected i.n. with 1.5 × 10⁷ colony-forming units per left lung developed progressive cervicofacial actinomycoses involving destruction of the jaw and posterior nasal regions (Fig. 1). Four of 23 nude mice died of pulmonary infection, with large abscesses developing within the tracheobronchial tree and extending into the lung. The remaining 16 nude mice remained free of clinical symptoms and, 3
months after infection, appeared healthy. Necropsy revealed no macroscopic evidence of disease, and *A. israelii* could not be isolated from these mice. A similar, but initially more acute, process occurred in the heterozygous (Nu/+) littermate mice. Of 17 Nu/+ mice that received $1.6 \times 10^7$ colony-forming units in the left lung, 4 died within 1 week after infection; however, the surviving mice completely cleared their infection. None of the heterozygous littermate mice developed cervicofacial lesions, nor did we observe large abscesses within the tracheobronchial region of any of the heterozygous (Nu/+) littermate mice.

Pulmonary clearance of i.n.-instilled *Actinomyces* is presented in Fig. 2. In this experimental series, none of the mice died as the result of adding approximately $1 \times 10^7$ colony-forming units in the left lung. Each point on the graph represents the mean of five mice for each group. It was found that within 24 h greater than 99% of *A. israelii* were cleared from the lung and at 72 h no organisms could be recovered from any of the 10 mice sacrificed at this time period. These data are in sharp contrast to those observed for i.n. instilled *N. asteroides* (2) and indicate that a functioning T-lymphocyte system is not essential for complete removal of *A. israelii* serotype I from the intact murine lung.

The 50% lethal dose values of i.n. instilled and i.v. injected *A. israelii* were determined by the standard Reed-Muench method (4). A minimum of 18 mice was used for each determination. The 50% lethal dose for i.n. inoculation is approximately $1 \times 10^6$ colony-forming units for both nude and heterozygous mice. This strain of *Actinomyces* was slightly more virulent when given i.v., and the 50% lethal dose by this route was approximately $6 \times 10^8$ colony-forming units for both groups of mice. None of the surviving mice had any evidence of infection when necropsied at 3 months after inoculation. Furthermore, we were unable to isolate *A. israelii* from any of these mice at this time period.

From these data it can be concluded that host resistance to disseminated infections with this strain of *A. israelii* is not dependent upon T-cells. Furthermore, T-cells appear not to be involved in clearance of these organisms from the lung. The data suggest, however, that T-lymphocytes may be involved in the resistance of the host to the localized, cervicofacial form of the disease that results from local contamination during intranasal administration, since approximately 30% of the nude mice infected in this manner developed either cervicofacial lesions or large abscesses of the tracheobronchial region. All of the nude mice did not develop this form of disease when given large numbers of *A. israelii*. Therefore, additional factors must be involved in the pathogenesis of Actinomycosis.

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